National Cancer Institute Spring 2014

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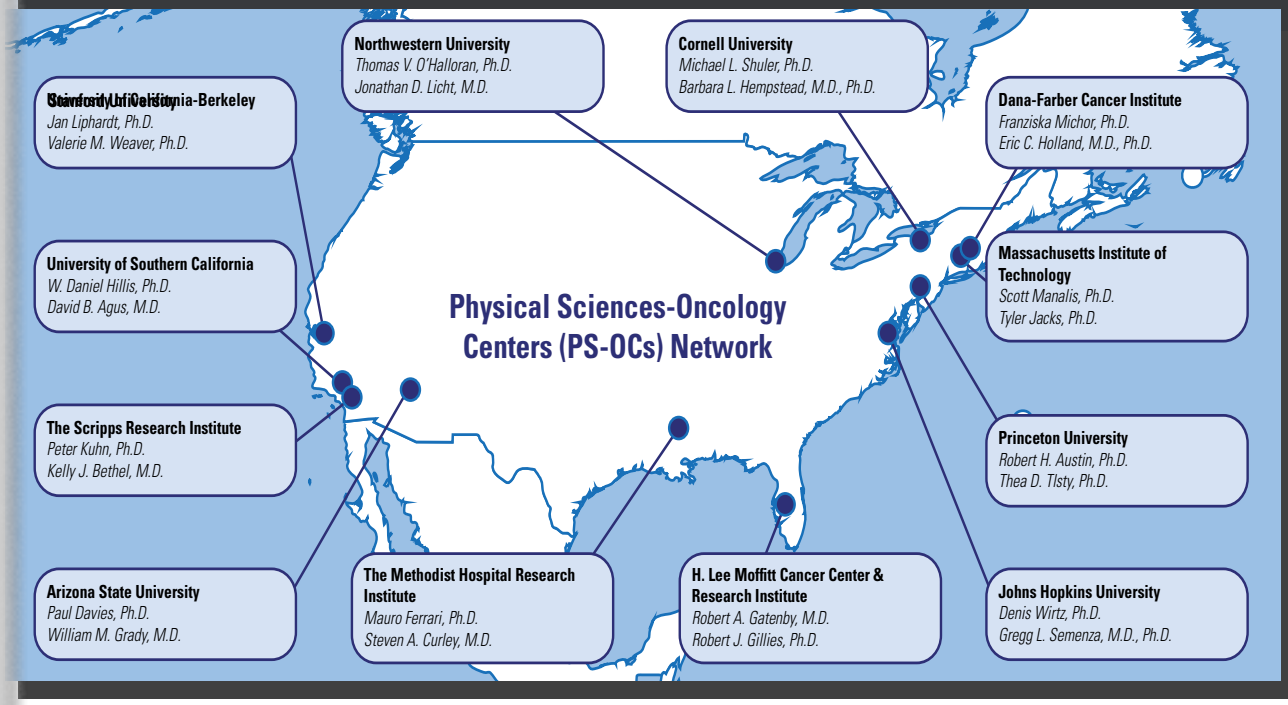
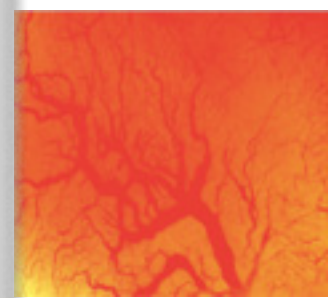
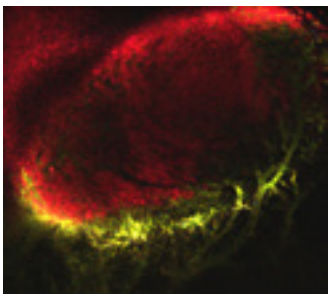
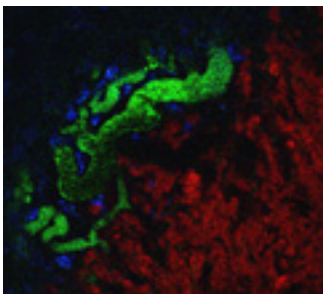
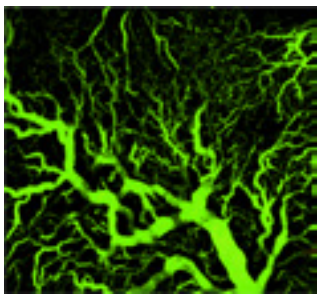
News from the Collaborative Network of Physical Sciences-Oncology Centers



In 2009, the NCI Physical Sciences in Oncology Initiative led to the establishment of the Physical Sciences – Oncology Centers (PS-OC) Program, a collaborative Network of twelve PS-OCs where physical scientists (e.g., mathematicians, chemists, physicists, engineers) team up with cancer biologists and oncologists to better understand the physical laws and principles that drive cancer emergence and progression.

This issue of Perspectives highlights some of the thought-provoking research projects taking place at each PS-OC. Some examples include: engineering white blood cells to kill off tumor cells found in the blood, using radio waves to increase the efficiency of chemotherapy, and using game theory concepts to model how resistance to chemotherapy develops in tumor cells. In "Voices of the PS-OCs," PS-OC members reflect on how the Program has influenced his or her career trajectory and approach to team science-based research since the Program's implementation.

A Novel Theory of Cancer Based on its Ancient Evolutionary Origins

by Paul Davies

Cancer is a disease of bodies. But in evolutionary terms, bodies are a relatively recent innovation.

For most of life's 4 billion year history, life has been unicellular, with the prime imperative of "replicate, replicate, replicate!"

About 1.5 billion years ago, in the Proterozoic era, some eukaryotic cells began to form colonies in which a new deal was struck. Reproduction was outsourced to specialized germ cells, while the remainder accepted programmed cell death, or apoptosis. Multi-celled organisms with bodies had finally evolved. Yet the primeval urge of unrestrained proliferation never went away. To police the new contract, many layers of regulation evolved to suppress cheating cells from defaulting to the natural state of runaway proliferation, or cancer.

Evidence that the evolutionary roots of cancer are very ancient comes from the observation that most multi-celled organisms are prone to it – mammals, fish, reptiles and even worms. In the new theory, cancer is regarded as a systematic and pre-programmed response to a stress or insult – a defensive reversion to cells' ancient core functionality.

The many distinctive hallmarks of cancer are, significantly, biological functions already performed in healthy organisms, most importantly in the processes of embryo development and wound healing. Cancer merely redeploys them in inappropriate ways.

The new theory posits that cancer creates niches in the body resembling conditions in the Proterozoic, for example, its low oxygen environment in which cells used an ancient mode of metabolism called fermentation. Sure enough, this is indeed a known hallmark of cancer. Another is that as cancer progresses in the body, it runs the arrow of evolution backward, jettisoning more recently evolved abilities first. Thus there should be a correlation between the way genes get expressed in cancer and the evolutionary ages of those genes.

To test the theory, we are combining the data from the Cancer Genome Atlas with genetic data from thousands of species to look for patterns. We think the key genes that drive cancer are generally at least hundreds of millions of years old, whereas many tumor suppressor genes, which often become non-functional in cancer, are more recent evolutionary innovations.

These ideas suggest a fundamentally different approach to therapy. Conventional therapy such as drugs and radiation mostly targets cells' proliferative ability. But proliferation is the most ancient defining characteristic of organisms, and life has had 4 billion years to learn how to combat such threats.

The new theory advocates targeting, not the principal strength of cancer – its proliferative prowess – but its weaknesses – that is, the vulnerabilities of cancer cells, which arise as they lose the more recently evolved regulatory functions.

These losses include DNA repair mechanisms to counter oxidative damage, a host of specialized efflux pumps to rid cells of toxins and the efficacy of the adaptive immune system to combat infections. All provide an opportunity to subject tumors to disabling challenges that can more easily be met by healthy cells. In this manner, cancer might be transformed from a lethal disease into a manageable chronic condition.

References: P C W Davies and C H Lineweaver, Cancer tumors as Metazoa 1.0: tapping genes of ancient ancestors. Physical Biology, Vol. 8, Number 1, February 2011

Anthracycline Drugs Enhance Nucleosome Dynamics

by Steven Henikoff

Doxorubicin is one of the most effective drugs used in cancer chemotherapy and along with related anthracycline compounds has been used in the clinic for four decades.

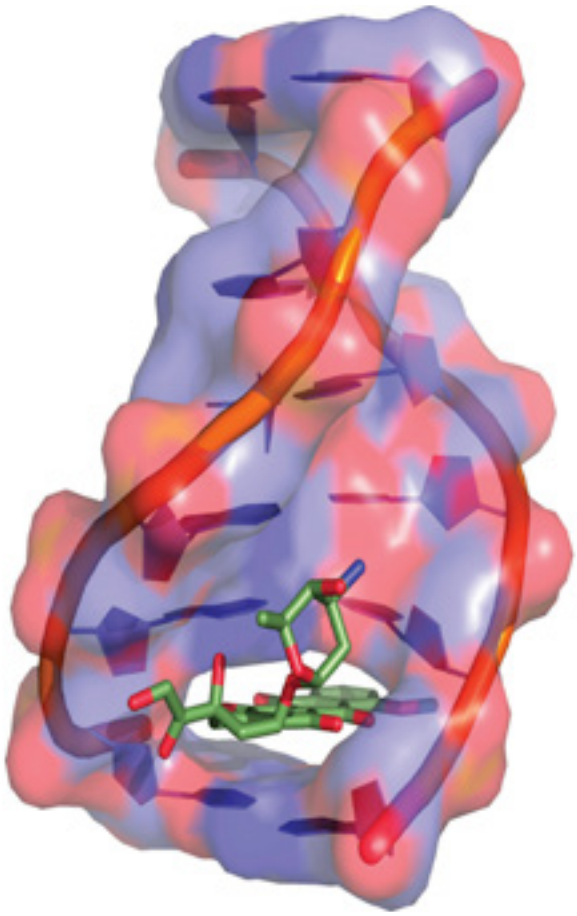
However, the anti-cancer mechanism of this key class of drugs remains unknown.

Anthracyclines intercalate between DNA bases **(Figure),** but whether intercalation is the key to the drug's efficacy is an open question.

Indeed, the most popular model for doxorubicin action does not invoke DNA intercalation, but rather posits that the drug traps an enzyme called topoisomerase II in the act of breaking DNA duplexes to relieve torsional strain.

The resulting unrepaired double-strand breaks are then thought to preferentially kill dividing cells.

However, aclarubicin is an intercalating anthracycline drug that like doxorubicin kills cancer cells but does not inhibit topoisomerase II, challenging this hypothesis.



Doxorubicin intercalates between DNA bases and pushes them apart.

A new idea for the anti-cancer activity of doxorubicin followed from a discovery by postdoctoral fellow Fan Yang.

Yang was studying the dynamic process of nucleosome turnover in the Henikoff lab of the ASU PS-OC.

Nucleosomes, which package DNA into chromosomes, are octamers of histone proteins that are wrapped by DNA. Nucleosomes are sometimes unwrapped and lost, especially around promoters of active genes.

Using a method that was recently developed in the Henikoff lab to measure nucleosome loss and replacement (turnover) genome-wide, Dr. Yang found that low levels of doxorubicin treatment enhanced nucleosome turnover around the start sites of actively transcribing genes.

Aclarubicin had a similar effect, which implied that enhancement of turnover is not, after all, a consequence of trapping topoisomerase II.

Rather, enhancement might be driven by intercalation of the drug, which pushes apart bases of (right-handed) DNA, thus transmitting positive torsion.

Positive torsion likewise occurs ahead of transcribing RNA polymerase, which unwraps (left-handed) nucleosomes, thus facilitating nucleosome turnover around active gene promoters.

Perhaps increased exposure of DNA from doxorubicin-enhanced nucleosome unwrapping causes DNA breaks that kill cancer cells?

Understanding the molecular effects of anthracycline drug treatment on nucleosome dynamics may provide new insights into doxorubicin dosing strategies and the design of better anti-cancer therapies, including those that combine new classes of drugs that target chromatin regulators with traditional chemo-therapeutic agents.

References:
Yang F, Kemp CJ and Henikoff S (2013) Doxorubicin enhances nucleosome turnover around promoters. Curr. Biol. 23:782-7.
Yang F, Teves SS, Kemp CJ and Henikoff S (2013) Doxorubicin, DNA torsion and chromatin dynamics. BBA Reviews on Cancer 1845:84-89.

A Tumor Can Be Deadly, but Metastasis is Deadlier

At the Cornell University Physical Sciences – Oncology Center (PS-OC), researchers in the labs of Mike King and Chris Schaffer are developing a novel approach to target circulating tumor cells (CTCs) that travel through the bloodstream after they have escaped a tumor.

These research groups have come up with a way to target CTCs before they metastasize – that is, circulate through the body to form tumors in other organs. Since metastasis causes 90% of cancer deaths, this work could have a significant impact.

Michael King, Cornell professor of biomedical engineering and the study's senior author, works with students Elizabeth Wayne and first author Michael Mitchell in lab.

Their approach starts with E-selectin, a protein found on endothelial cells that make up blood vessels. E-selectin can bind the CTCs that travel throughout the bloodstream and lymph nodes. Previous work done in the King lab had investigated E-selectin's role in CTCs' adhesion to the walls of blood vessels. This adhesion is the critical first step in the cells' escape from the circulatory system and into tissue, where they can seed and begin to form tumors.

The King lab has recently developed liposomes (artificial vesicles composed of lipids) covered with both E-selectin and the TRAIL (TNF-related apoptosis-inducing ligand) protein, which exclusively causes cancer cells to undergo programmed cell death. When these modified liposomes bind to white blood cells circulating throughout the bloodstream, they transform the white blood cells into "unnatural killer cells" that target circulating cancer cells without damaging healthy cells. Upon injection of the modified liposomes, the number of circulating tumor cells drops precipitously, unlike in the presence of either E-selectin or TRAIL alone.

The novel approach of using white blood cells to target CTCs was fueled by the interdisciplinary nature of the PS-OCs.

"This paper is the culmination of our involvement in the PS-OC program since 2009," says King. "It wouldn't have happened if [Schaffer] and I were not part of the Center."

This work presents an exciting new method of potentially preventing metastases in cancer patients. To date, the work has focused on in vitro tests with human blood and in shorter-term mouse model experiments.

Now the labs are focusing on using mouse models that spontaneously form tumors and metastases to test the effectiveness of this treatment on metastases as they develop. The researchers are also examining the ability of these cells to target CTCs circulating through the lymphatic system.

Results of this study have recently been published in the *Proceedings of the National Academy of Sciences*, and the work has been picked up by a number of news outlets including BBC News.

References: Michael J. Mitchell, Elizabeth Wayne, Kuldeepsinh Rana, Chris B. Schaffer, and Michael R. King. PNAS 2014 111 (3) 930-935

From Clean Room to Bedside—Microfabricated Devices used in a New Clinical Trial

A microfluidic device designed and fabricated at the Cornell University PS-OC is being used as part of a new clinical trial.

"We want to use microtechnology to access the most important cancer cells inside patients' bodies, and use that in concert with modern surgical and pharmaceutical techniques to improve patient care," says Brian Kirby, Cornell Professor in mechanical engineering and PS-OC researcher.

The device, called a Geometrically Enhanced Differential Immunocapture (GEDI) chip, is designed to trap circulating tumor cells (CTCs) from blood. As these tumor cells are typically present in concentrations as low as one per billion normal cells, efficient and accurate trapping is key to an accurate metric.

The chip consists of a small chamber filled with arrays of posts that are 100-150 microns in diameter—around the diameter of a human hair. The posts are precisely arranged to cause the larger CTCs to bump into the posts while the smaller cells in healthy blood pass through smoothly when a blood sample flows into the device. The posts are covered with antibodies that bind to specific cancer cells, so by encouraging the cancer cells to bump into the posts, researchers increase the likelihood of trapping the CTCs.

Professor Kirby, PhD student Fredrik Thege, and Research Associate Shalu Suri examine a GEDI microdevice.

Once trapped, these cells can be counted to determine the effectiveness of current treatment, or can be exposed to chemotherapeutic drugs for testing.

The GEDI chip is being used as part of an ongoing multi-institutional clinical trial led by a team including PS-OC members Brian Kirby, David Nanus, and Evi Giannakakou as well as Scott Tagawa, Emmanuel Antonarakis and Mario Eisenberger.

The trial is measuring the efficacy of two cancer drugs (Jevtana and Taxotere) on prostate cancer patients while also evaluating the GEDI device's ability to predict which drug will work better in patients. If effective, the trial may lead to more efficient tailoring of cancer treatment to a particular patient.

The Kirby lab is also tailoring the GEDI chip to other cancers and applying the results of the current studies to personalizing treatment and early detection of cancer.

References: Kirby BJ, Jodari M, Loftus M, Gakhar G, Pratt ED, Chanel-Vos C, Gleghorn JP, Santana SM, Liu H, Smith JP, Navarro VN, Tagawa ST, Bander NH, Nanus DM, Giannakakou PA. PLoS ONE, 7(4): e35976, 2012.

Revamped Radiation Treatment Schedule for Common Form of Brain Cancer Can Extend Survival

A new study by researchers at Dana-Farber Cancer Institute and other organizations has shown that an altered radiation treatment schedule for the most common and lethal form of brain cancer extended the survival period of mice with the disease – suggesting that it may be able to do the same for human patients.

The study, published online by the journal *Cell*, involves glioblastoma, a brain malignancy diagnosed in almost 3,000 people each year in the United States. Because the research involved mice, the study does not recommend a specific new schedule for human patients, but the findings demonstrate that modifying the standard administration schedule of radiotherapy can make the treatment more effective, the authors say.

The research is based on a new understanding of how glioblastoma cells respond to radiation therapy, and how that response toughens them against the ravages of radiation.

"There have been many attempts over the years to develop a more effective radiation therapy schedule for patients with this disease, but none has proven superior to the standard approach, which has now been in use, essentially unchanged, for more than 50 years," says Franziska Michor, PhD, Principal Investigator of the PS-OC at Dana-Farber.

"An array of recent advances in the understanding of the basic biology of glioblastoma led us to try a fresh approach to the problem."

Glioblastoma is a malignant brain cancer that is more common in older people than in young and affects more men than women.

Treatment generally consists of surgery, chemotherapy and radiation therapy. While the clinical management of the disease can extend patients' lives, it is currently incurable: the median survival of treated patients is about 15 months.

The new study was spurred by advances in three Areas: the discovery of different subtypes of glioblastoma based on the abnormal genes within their cells; the development of better mouse models of the disease in humans; and the discovery that some of the cells in glioblastoma tumors are similar to stem cells – immature cells that can withstand radiation therapy better than most glioblastoma cells.

Recently, scientists discovered that radiation therapy can cause glioblastoma cells to "de-mature" - to revert to a state where they're more like stem cells, and more resistant to being killed by radiation.

"There's a dynamic equilibrium within glioblastoma tumors in which cells are shifting between an immature and mature state," Michor says.

"We set out to see if we could use our understanding of this process to enhance the effectiveness of radiotherapy."

For the current study, Michor and her colleagues focused on a subtype of glioblastoma in which the protein PDGF (platelet-derived growth factor) induces abnormal signaling within normal cells. Such tumors account for about 30 percent of all glioblastomas.

The research team developed a mathematical model of the effect of radiation on glioblastoma cells – how quickly it prompts the cells to become more stem-like, how likely it is to kill cells, and how long these and other processes take. They then used the formulas to devise a treatment schedule that would, in theory, prolong survival.

"In radiotherapy, timing is everything," Michor relates.

"Irradiating too frequently would increase side effects and toxicity. Irradiating too infrequently would give the tumor cells too much opportunity to grow."

The standard radiotherapy schedule for patients with glioblastoma is 2 Grays (or Gy, a standard unit of absorbed radiation) per day, five days a week, for six weeks. The researchers identified a schedule for

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delivering a total of 10 Gy that was predicted to produce better results in mice. This optimum schedule administers the same total amount of Gy, but in a different temporal order.

They tested the new schedule in mice with glioblastoma and found that, indeed, they survived longer than similar mice treated under the standard schedule.

The improvement was attention-getting: mice treated under the standard schedule survived a median period of 33 days, compared to 50 days for the mice treated under the new schedule.

Because human glioblastoma patients usually receive a chemotherapy drug in conjunction with radiotherapy, and because the time scales of treatment response might be different from those in mice, the new schedule might not have an equally beneficial effect in patients, the authors' state, but studies are under way that more closely replicate the conditions of human treatment.

"Our model demonstrates that a revised dosing schedule can increase the number of stem-like cells in glioblastoma tumors and still slow the overall growth of the tumor and delay the time until tumor growth recurs," Michor remarks.

"The fact that we've accomplished this in animals raises the hope that we can achieve similar results in humans."

Franziska Michor, PhD, principal investigator of the Physical Sciences – Oncology Center at Dana-Farber is co-senior author of the study with Eric Holland, MD, PhD, of the Fred Hutchinson Cancer Research Center.

The co-lead authors of the study are Kevin Leder, PhD, of the University Minnesota and Ken Pitter of Memorial Sloan-Kettering Cancer Center. Co-authors are Quincey LaPlant, PhD, and Timothy Chan, MD, PhD, of Sloan-Kettering, Dolores Hambardzumyan, PhD of the Cleveland Clinic; and Brian Ross, PhD, of the University of Michigan.

Improving Therapy Options for Patients

We are a multi-disciplinary group composed of physicians, physicists, mathematicians, engineers and biological scientists, all focused on one goal: “to contribute to the prevention and cure of cancer”. This sentence is reproduced on walls and signs all over the Cancer Center to continuously remind us of our ultimate mission.

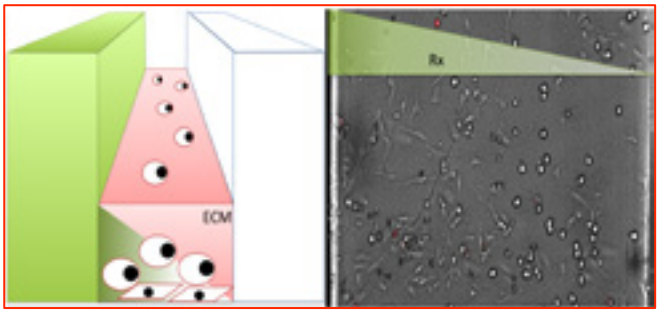

FIGURE 1: *Each microfluidic chamber is composed of two side reservoirs, and one center observation chamber. Myeloma and stromal cells are loaded in the observation chamber simultaneously, suspended in collagen (left). After 24h of chemotherapy exposure, cells on the highest concentration are dead, while those in lowest concentration survive (right).*

In the last three years, with funding from the PS-OC Program, other programs at the NCI, and the State of Florida, we have undertaken an ambitious project: the use of mathematical modeling to predict therapy response in cancer patients, and ultimately use this information to decide the best therapy for each patient.

Cancer is a dynamic disease, a moving target. It is composed of a multitude of individual cells that are slightly different from each other, and are continuously changing and altering their environment.

No mathematical model, no matter how well elaborated, can adequately describe an individual cancer patient, unless it uses data obtained from that very patient, at that very moment in time.

Our choice of cancer was multiple myeloma (MM). This cancer develops in the bone marrow, mainly in elders (>65yo), and although treatable, all patients eventually relapse: that is, their tumors return and no longer respond to therapy. MM cells are the counterparts of plasma cells, which are white blood cells that secrete antibodies; as such, MM cells produce one specific antibody that is secreted in blood and detectable in urine.

If the blood concentration of this antibody roughly doubles, for instance, this indicates that the number of MM cells has doubled.

Another property of MM cells is that they are significantly more resistant to therapy when they are embedded in the bone marrow and in direct contact with the healthy cells of the marrow (stroma).

Unfortunately, conducting experiments in the laboratory with MM cells in combination with stroma is a cumbersome and costly task. For this reason, most studies in the past and present consist of MM cells floating in flasks with liquid “media” that primarily serves as a source of nutrition for the cultured cells.

In order to achieve the most realistic experimental setting possible while still generating quantitative and detailed data for our mathematical models, our group has developed an experimental platform (**Figure 1**) that consists of mixing MM cells fresh from a patient biopsy with patient stroma in a collagen gel droplet.

The mix is transferred to microscopic chambers (with volumes in the order of a millionth of a liter), where cells are treated with a panel of chemotherapeutic drugs and continuously imaged by a digital microscope at 5- to 10-minute intervals.

A program developed for this purpose uses these images to detect live and dead cells at any given moment during the experiment (Khin et al., 2014).

Our next step was to incorporate an important and dynamic element of tumor response to therapy: the microenvironment of the bone marrow. For this purpose we have studied bone marrow biopsies from MM patients at different stages of treatment.

We used antibodies to detect myeloma cells as well as elements of the bone marrow such as the stroma, which may serve to protect these cells, and adhesion molecules that help cells stick to their surroundings.

By super-imposing the signals from these antibodies, we were able to determine the locations of these different elements.

Additionally, we were able to quantify the proportion of MM cells in each patient that were located in niches where they are protected from therapy vs. those where MM cells must stand unprotected on their own.
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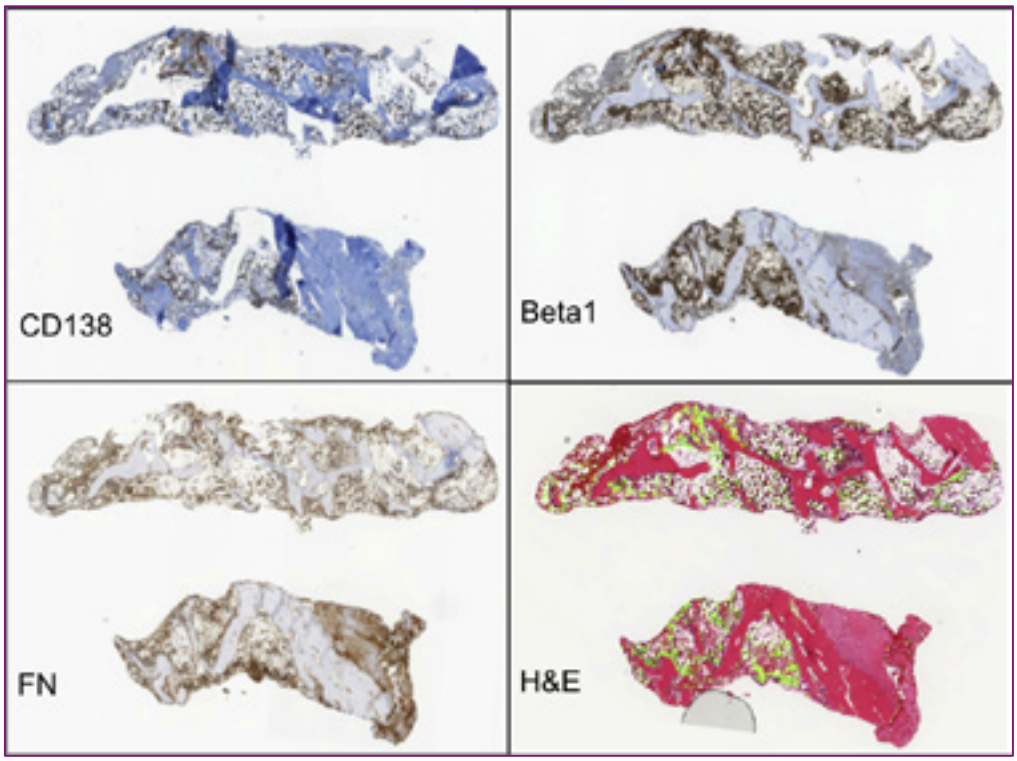

FIGURE 2: *Cell adhesion is a major mechanism of chemoresistance in MM. Bone marrow biopsies were stained for CD138 (MM marker), integrin beta1, and fibronectin. Digital image analysis was used to quantify co-localized signals as the intensity of environmental protection (green).*

The first group would thus have a higher chance of surviving treatment than the second (**Figure 2**).
By combining these two elements, *i.e.*, the "seed" (cancer cell) and the "soil" (the bone marrow), we have been investigating how these patient-personalized models of drug sensitivity can be used to simulate how each patient would respond to therapy (**Figure 3**).

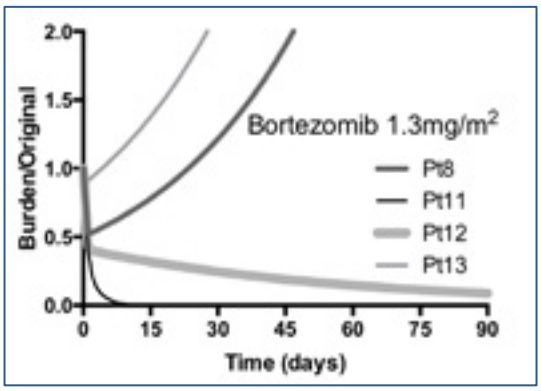

FIGURE 3: *The sensitivity of primary cells from four MM patients (pt8, pt11, pt12, and pt13) to the chemotherapeutic drug bortezomib was tested ex vivo and extrapolated to a clinical regimen of 1.3mg/m^2 bortezomib. Simulations suggest that this regimen would induce a complete response in patients pt11 and pt12, a partial response in patient pt8, and no response in patient pt13.*

We expect that these models will also be used to choose from different drug combinations as well as investigate alternative therapeutic regimens that are tailored for each patient, focusing on quality of life and overall survival.

References: Khin ZP, Ribeiro ML, Jacobson T, Hazlehurst L, Perez L, Baz R, Shain K, Silva AS. A preclinical assay for chemosensitivity in multiple myeloma. Cancer Res. 2014 Jan 1;74(1):56-67. doi: 10.1158/0008-5472.CAN-13-2397. PMID: 24310398, PMCID: PMC3915502 [Available on 2014/1/1]

Cell Migration in Confined, Unconfined Spaces Regulated Differently

by Mary Spiro, INBT science writer

A team led by Konstantinos Konstantopoulos, Professor of Chemical and Biomolecular Engineering and Department Chair, investigated how physical confinement impacts cell movement. In order to assess this, the investigators made use of a device called a micro-channel array in which cells were placed into a confined microenvironment made up of micro-channels of various widths.

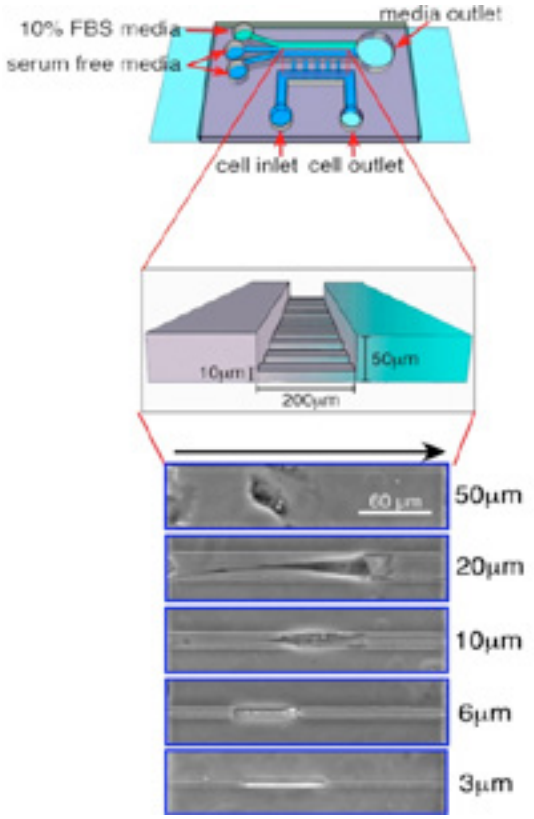

Illustration of the micro-channel array device.

With this tool, the Konstantopoulos team found that signaling through α4β1 integrin, a molecule found on white blood cells that facilitates their binding to blood vessel walls, mediates cell migration through both unconfined and confined spaces. They also observed that crosstalk occurs between Rac1, a molecule that regulates processes such as cell growth, and myosin II, a motor protein involved in muscle contraction. This crosstalk controls the migration of fibroblasts (precursor cells that eventually form the material providing structural support to tissues) in this microenvironment. Wei-Chien Hung, a pre-doctoral student in the Konstantopoulos lab, was the lead author on this study.

Experiments using the micro-channel array revealed that when a cell is crawling through a spacious channel (50 μm wide), it slides through unhindered using a movement style regulated by Rac1. A narrower channel (10 μm wide) constrains the cell. When the width is confined to a very tight space (3 μm wide), the cell must squeeze itself to fit through, and its movement is driven by myosin II. It was also determined that two variations of Myosin II, MIIA and MIIB, are required for confined and unconfined migration, respectively.

"This study shows that cells are more plastic than previously thought, and that physical microenvironment alters cell migration mechanisms," Konstantopoulos said.

Since most cancer deaths are caused from metastasis (tumor cell spread to distant sites) and not from the primary tumor alone, Konstantopoulos and his lab group are working to better understand the metastatic process so that effective preventions and treatments can be established.

"Distinct signaling mechanisms regulate migration in unconfined versus confined spaces," published in *Journal of Cell Biology, August 26, 2013* can be found here: http://www.ncbi.nlm.nih.gov/pubmed/23979717.

Additional authors included Shih-Hsun Chen, Colin D. Paul, Kimberly M. Stroka, Ying-Chun Lo, and Joy T. Yang, all of Johns Hopkins University

Paradigm Shifting From 2D to 3D Cell Analysis

By shifting the emphasis from studying cells growth and development in 2D, as on a Petri dish, to 3D, as is likely to be the case in the body, Johns Hopkins PS-OC researchers in the Denis Wirtz Laboratory are making bold new discoveries about cancer spread. For example, they found that the Arp2/3 complex (a complex of proteins responsible for helping cells "crawl" in 2D environments) creates dendritic, or arm-like, protrusions that move cells through a 3D environment. The contribution of Arp2/3 and several associated proteins was significantly different when comparing 2D to 3D environments. *(Giri et al, 2013).*

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Working with Benjamin Schafer in civil engineering, Wirtz Lab researchers built a 2D computational "form-finding" model that served to simulate the natural state of the actin-cytoskeleton network, which is a network of proteins that gives cells their shape and structure. The investigators employed this model to discover how stiffness within a cell's environment can contribute to cell shape and ability to move. They are now working on a 3D form-finding model. *(Gong et al, 2013).*

Although revisiting cell biology questions that have been addressed in 2D but not 3D will be more complicated, Wirtz says it must be done if science is to ascertain the true nature of metastasis and find drugs to treat cancer.

He believes there may be drugs we missed because of poor performance in 2D screenings that may have a completely different outcome when put in the context of a physiologically relevant 3D environment.

PS-OC Summer Interns

Johns Hopkins PS-OC hosted two undergraduate summer interns who conducted research in center-affiliated laboratories for 10 weeks. At the conclusion of their researcher experience, they joined several dozen other summer researchers to present their work at a collaborative poster session at the Johns Hopkins School of Medicine.

One of the two interns was Cameron Nemeth (below), a student from the University of Washington who worked in the laboratory of Hai-Quan Mao, Professor of Materials Science and Engineering. Nemeth investigated how Schwann cells, the essential support cells of the peripheral nervous system, can provide migration cues by delivering different gradients of proteins called neurotrophic factors that play a role in neuron growth. Nemeth studied the direction and speed of Schwann cell migration by establishing a neurotrophic factor gradient and guidance system using a special material made up of nanofibers that had been developed in the Mao lab.

Schwann cells are important to nerve development and regeneration, and transplantation of Schwann cells has helped in cases where nerves have been injured. However, having the ability to control the migration of Schwann cells would improve outcomes after transplantation.

Nemeth's work will add to understanding the processes surrounding nervous tissue repair.

Cameron Nemeth worked in the Mao Lab

Solving Chromosomes' Structure: Scientists Find That Loops of DNA are Key to Tightly Packing Genetic Material for Cell Division

by Anne Trafton

Scientists first discovered chromosomes in the late 1800s, after the light microscope was invented.

Using these microscopes, biologist Walter Flemming observed many tightly wound, elongated structures in cell nuclei. Later, it was found that chromosomes are made from DNA, the cell's genetic material.

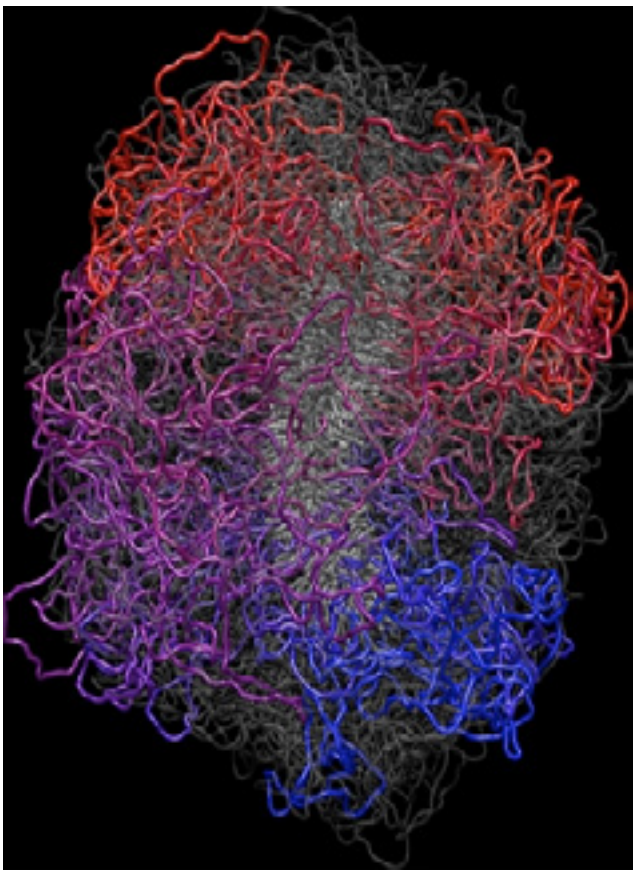
Since then, scientists have proposed many possible ways that DNA molecules might fold into three-dimensional (3-D) condensed chromosomes. Now, researchers at the MIT Physical Sciences-Oncology Center (MIT PS-OC) and the University of Massachusetts Medical School have obtained novel data on the 3-D organization of condensed human chromosomes and built the first comprehensive model of such chromosomes.

In this model, DNA forms loops that emanate from a flexible scaffold; the loops are tightly compressed along the scaffold.

"This is a very efficient way of packing DNA material," says Leonid Mirny, an associate professor of health sciences and technology and physics at MIT and a senior author of a paper describing the findings in the Nov. 22, 2013 edition of *Science*.

This condensed state, seen only when cells are dividing, allows cells to neatly separate and distribute their chromosomes so that each daughter cell receives the full complement of genetic material.

At all other times, the chromosomes are more loosely organized inside the cell nucleus.



A model of human mitotic chromosome is shown. The chromosome is composed of a flexible scaffold (gray) and a system of loops (red, magenta, blue). Illustration: Imakaev M., Fudenberg G., Naumova N., Dekker J., Mirny L.

Layers of Structure

Chromosomes are complex molecules with several levels of organization, allowing cells to cram 2 meters of DNA into a nucleus that is only one hundredth of a millimeter in diameter. Long strands of DNA wind around proteins called histones, giving rise to a "beads on a string" structure. Several models have been proposed to explain how those strands of millions of beads are arranged inside tightly packed chromosomes.

"There is no shortage of models of how DNA is folded inside a chromosome," says Mirny, who is a member of MIT's Institute for Medical Engineering and Sciences at the MIT PS-OC.

"Every high-school biology textbook has a drawing of chromosomes folding. If you look at these drawings you might get the impression that the problem has been solved, but if you look carefully you see that all these drawings are very different."

To help determine which model is correct, the Mirny and the Dekker labs have collaborated to use a technology developed in Job Dekker's lab at the University of Massachusetts Medical School called Hi-C, which performs genome-wide analysis of the proximity of genomic regions.

This reveals the frequency of interaction for every pair of regions in the entire genome. The challenge, however, lies in generating an overall chromosome structure based on Hi-C data.

"Given a 3-D structure, it is straightforward to find all contacts; however, reconstructing 3-D structures from contact frequencies is much more difficult," says Maxim Imakaev, a member of the MIT PS-OC who was also involved in this research.

(Continued on next page)

In 2009, researchers including Imakaev, Mirny, and Dekker used Hi-C to demonstrate that during most of a cell's life, when it is not dividing, DNA is organized as a fractal globule, in which DNA is not tangled or knotted. Hi-C also showed that regions with more active genes tend to cluster together in easily accessible compartments, and unused regions form more densely packed clusters.

The organization of each chromosome varies among cell types, because every type of cell uses different sets of genes to carry out its functions. This means that each chromosome acquires a specific 3-D organization depending on which genes a cell is using.

Chromosomes During Cell Division

In the new paper, the MIT PS-OC researchers found that as cells begin to divide, chromosomes are completely reorganized.

First, all chromosome-specific and cell type-specific patterns of organization, which are necessary for gene regulation, disappear.

Instead, all chromosomes are folded in a similar way as cells begin to undergo cell division, or mitosis. However, the chromosomes do not form the exact same structure every time they condense.

***"Unlike proteins, which fold into very defined structures, a chromosome forms a completely different condensed object every time,"* says Geoff Fudenberg, a member of the MIT PS-OC who was also involved in this research. *"The individual regions of the genome appear similar macroscopically but can be folded in very different ways in different cells,"* Fudenberg adds.**

The Hi-C technique *"provides a modern day molecular microscope, with the power to see inside these bodies and elucidate their principles of organization,"* wrote Nancy Kleckner, a professor of molecular and cellular biology at Harvard University, in a perspective article accompanying the *Science* paper. The researchers *"combine chromosome conformation capture with polymer physics simulations to provide a new, yet satisfyingly familiar, view,"* she wrote.

The researchers believe that two stages are required to achieve the loop-on-a-scaffold structure: First, the chromatin forms loops — each of which contains about 80,000 to 120,000 DNA base pairs — radiating out from a scaffold made of DNA and some proteins.

Then, the chromosome compresses itself along its central axis, where the scaffold is located.

While molecular details of the second stage remain mysterious, scientists have a good guess for what might be responsible for the first stage of chromosome folding: investigators at the Northwestern University PS-OC recently proposed that proteins called condensins drive chromosome condensation by latching on to the DNA and extruding loops.

To test this hypothesis in greater detail, the MIT team is now collaborating with these researchers.

Beyond characterizing condensed chromosomes, this study also opens the door for future work to understand mechanisms of chromosome condensation, cell memory, and epigenetic cell reprogramming.

Geoff Fudenberg, MIT PS-OC

Physical Transport Properties Could Predict Outcomes for Patients with Cancer

by Eugene Koay, M.D., Ph.D. (University of Texas MD Anderson Cancer Center)
Vittorio Cristini, Ph.D. (University of New Mexico)

The clinical outcomes from pancreatic cancer have not improved for two decades, despite advances in drugs and technology. One of the reasons for the poor outcome from this disease is that pancreatic tumors are typically composed of dense, fibrotic tissue that is difficult for chemotherapy drugs to penetrate.

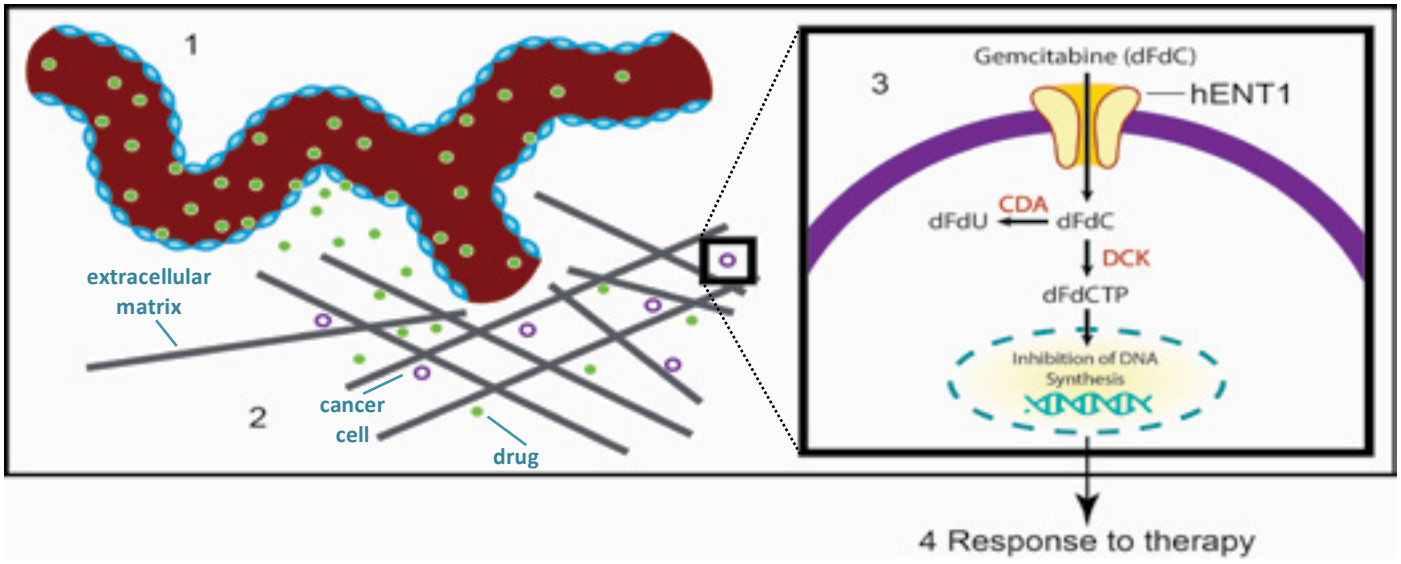
This complication decreases the chances of being able to kill the cancer cells. Our team, composed of physicians (surgeons, diagnostic radiologists, pathologists, anesthesiologists, medical oncologists, and radiation oncologists), basic scientists, engineers, and mathematicians, strives to understand the drug delivery problem in humans with pancreatic cancer and other cancer types. Our collaborative approach applies principles of physics to assess human tumors. We also conduct clinical trials to measure efficiency of drug delivery in human tumors and outcomes of patients.

The results in pancreatic cancer show how the physical transport of chemotherapy—and the subsequent outcome of the patient—can be described by quantitative analysis and mathematical modeling of routine diagnostic imaging (computed tomography, or CT scans).

We also demonstrate how physical transport processes at multiple scales (such as the blood vessels, fibrotic matrix surrounding cancer cells, and transport proteins found in the cell membrane depicted in the figure) need to be considered to fully understand the drug delivery process. These data suggest that we can use physical principles to identify patients with pancreatic cancer who may benefit from certain types of therapies. *[Koay et al., J Clin Investigation 2014*]. We have also applied similar approaches to other cancers.

Interestingly, we have found that our physics-based approach with regard to pancreatic cancer is part of a more general theory of tumor drug response, which we have now applied to cancers of the brain, liver, breast, and lymph glands *[Cristini and colleagues, PNAS 2013, ACS Nano 2013, PLOS One 2013].*

These exciting results support the idea that drug response to chemotherapy is strongly influenced by physical transport phenomena. Our mathematical models can accurately describe these processes, and the results from our work could lead to new ways to overcome the barriers to drug delivery, which could enhance the response to the drugs and improve outcomes.



The physical process of drug delivery: *We developed ways to quantify the drug delivery process in humans with pancreatic cancer. Our method describes how the drug (1) moves out of blood vessels, (2) through the surrounding cellular matrix, and (3) across the cancer cell membrane. The amount of drug delivered is ultimately thought to influence (4) the response to therapy and the patient's outcome. (From Koay et al., JCI 2014).*

Knocking Down Transport Barriers: Non-Invasive Radiofrequency Therapy Enhances Uptake of Chemotherapy Agents into Breast Tumors

by Stuart James Corr, Ph.D., Baylor College of Medicine, Rita E. Serda, Ph.D., Baylor College of Medicine and Steven A. Curley, M.D., Baylor College of Medicine

Radio waves, just like those you tune into using your car radio, are all around us. Although these waves are mostly low-power and do not affect normal human physiology, concentrated high-power radio waves can penetrate deep into the body, causing a gradual increase in tissue and organ temperature.

This temperature increase is caused by the differential dielectric values of the human body (dielectric in this case refers to the physical variables associated with the way materials absorb and convert electrical energy to heat).

In regards to cancer therapy, the idea is simple – exposing tumors to radio waves for a certain period of time will raise the temperature of the tumor to lethal levels (hyperthermia), while at the same time leaving the normal neighboring tissues unaffected.

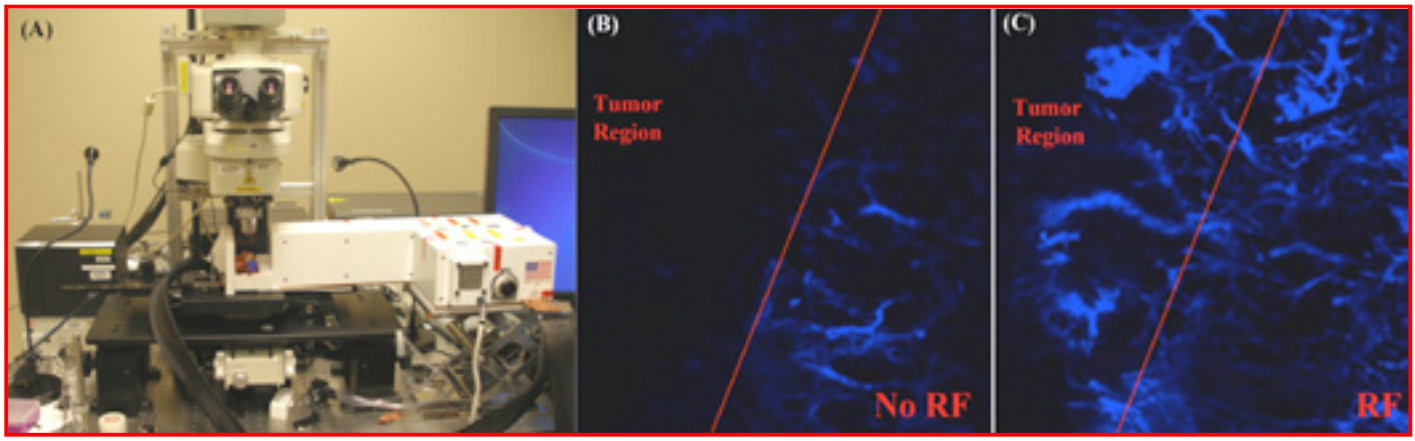
Recent progress in Dr. Steven Curley's lab at Baylor College of Medicine has shown that radio waves alone are capable of controlling tumor growth in mice with liver and pancreatic cancer.

This was found to be due to tumors having larger dielectric constants than normal tissues, which in essence allowed the tumor itself to be a targeting agent – sucking up and converting electrical energy to heat even more so than normal tissues.

In a bid to actually visualize the effect of radio waves on cancerous and normal tissues, Dr. Stuart J. Corr built a portable radiofrequency (RF) system that can be retrofitted to an Intravital Microscopy system (Fig. 1A), allowing imaging of cancerous lesions under RF exposure in real-time. Working with Dr. Rita E. Serda, Dr. Curley and his group examined the effect of RF on mice bearing breast tumors grown in the mammary fat pads by using a variety of fluorescent tracers to detect any potential differences in normal vs. cancerous tissue.

As can be seen in Figure 1B, there is a clear-cut 'barrier' that limits the flow of drugs into the tumor blood vessels. This barrier hinders the uptake of drugs, bio-molecules, and chemotherapy agents into the tumor. However, after exposure to RF for 5 minutes (Fig 1C), the group was amazed to see that they could effectively 'cross' and 'knock through' this barrier, thereby allowing for an increase in uptake of fluorescent dyes such as dextran and albumin. Ongoing work and future plans will optimize the conditions for maximum small-molecule, chemo-agent, and nanoparticle uptake.

Figure 1:
(A) *IVM system retrofitted with a portable RF system.*
(B) *Breast tumor imaged using albumin fluorophore (blue). No albumin gets across the transport barrier (red line) into the tumor region.*
(C) *RF exposure for 5 min. overcomes this transport barrier and allows enhanced uptake of albumin into tumor.*



Chromatin and Cancer

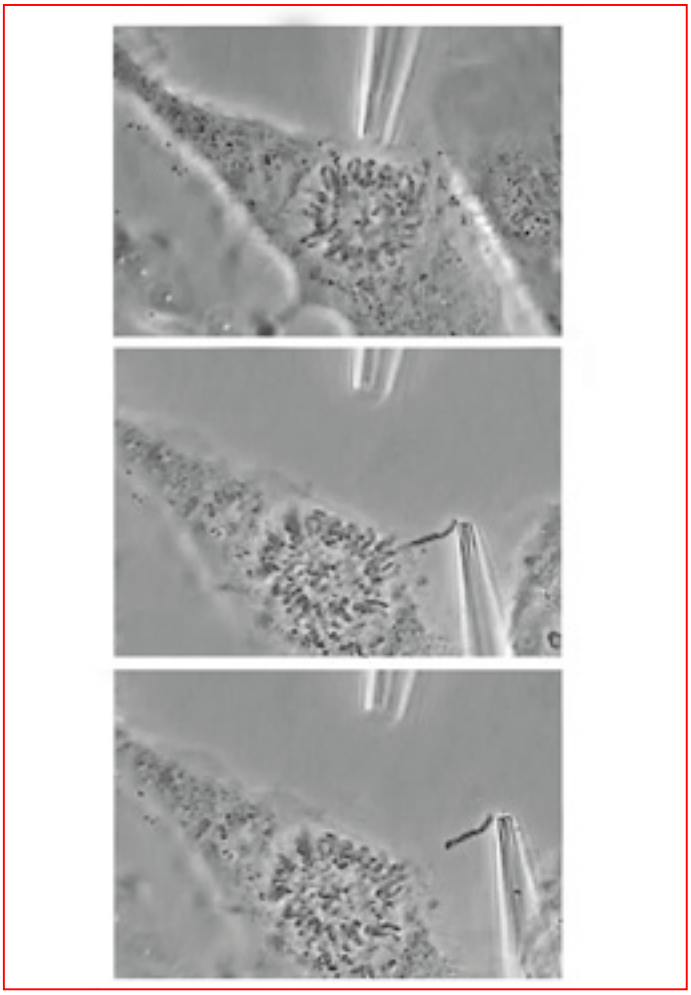
It is widely appreciated that the DNA in genes, the units of inheritance that reside within chromosomes, is the biological repository for information that dictates how cells look and behave.

Changes to this DNA are widespread in tumors and have been extensively catalogued, with some of them linked to malignancy. DNA is not the only component of genes and chromosomes, however.

Rather, the DNA is wrapped around a set of eight proteins, called histones, to form structures called nucleosomes. This is considered to be the first level of packaging of DNA.

The nucleosomal DNA further forms a complex with a variety of different proteins such that the final product, termed chromatin, is highly compacted and intricately folded and organized.

Scientists now recognize that identical DNA sequences can be interpreted differently by a cell, depending on the nature and modifications of the proteins associated with those sequences in chromatin.



Grabbing onto chromosome for stretching

This concept has given rise to a field of study termed “epigenetics” and has prompted many cancer researchers, including members of the Northwestern PS-OC, to determine whether differences in the components of chromosomes may be partially responsible for the differences in the appearance and behavior of cancer cells.

Although great advances have been made in understanding the properties of individual genes, there is a surprising paucity of basic information about chromosomal architecture in general, even in normal cells.

There are many outstanding questions:

What is the complete repertoire of proteins in chromosomes? How are these proteins organized relative to the DNA and to one another? What is responsible for the structural integrity of chromosomes?

This fundamental information is needed to determine how chromosomal structure is altered in malignant cells, how such alterations affect the read-out of genetic information, and how these structural alterations contribute to tumor growth.

Professor John Marko, a biophysicist, and his colleagues are using physical sciences-based approaches to examine how the chromatin in cancer cells differs from its counterpart in non- malignant cells.

One of the approaches used measures the force necessary to mechanically stretch chromosomes.

If these forces differ significantly between normal and cancer cells, the Marko lab will explore whether the differences in cancer cells are related to the nature, amount, or organization of specific proteins that are essential for overall chromosomal structure.

Their initial focus is on the condensin family of chromosomal proteins that cells require for chromosomal packaging and assembly.

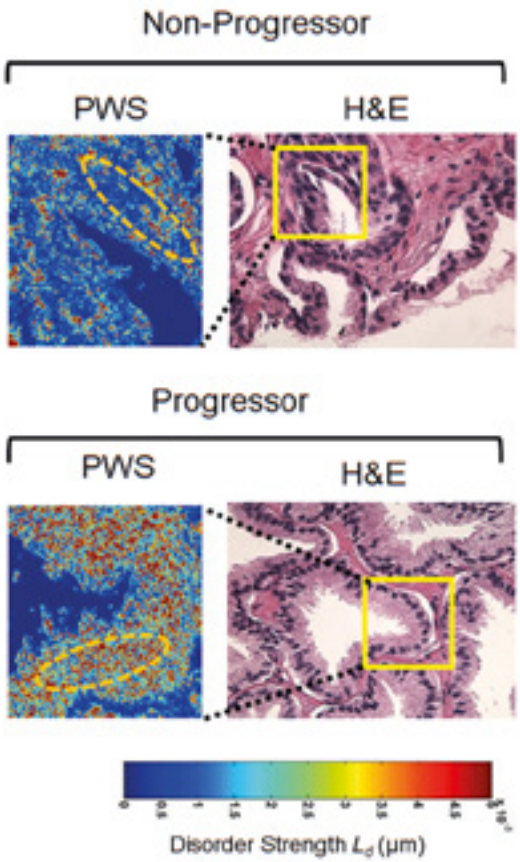
When chromosomes are artificially depleted of condensins, the chromosomes become less stiff. Marko and colleagues have developed a way to

(Continued on next page)

visualize the distribution and organization of condensins in chromosomes in hopes of gaining insights into the mechanism by which condensins contribute to chromosomal architecture.

Chromosomal breakage is commonly seen in cancer cells and seems to be highly associated with malignant behavior.

Early in the twentieth century, scientists, using crude microscopes that magnified the cell nucleus, recognized that the chromatin in cancer cells looked different than it did in normal cells. This was well before the discovery of genes and DNA, so they did not understand what this observation signified.



Histologically normal prostate epithelium; Stained (H&E) and PWS

Using a much more sensitive and sophisticated optical method called Partial Wave Spectroscopy (PWS) that is based on the scattering of light by individual cellular components, Professor Vadim Backman (Biomedical Engineering, Northwestern University) and his colleagues have been able to detect changes in the light-scattering properties of cells at the earliest stages of cancer development.

Importantly, these changes can be detected in cells that reside at a considerable distance from existing tumors but show no other signs of malignancy, indicating that PWS may be used as a non-invasive early detection tool for certain tumors (colon, pancreas, and lung). Dr. Backman has collaborated with scientists across multiple fields to determine the molecular basis for the differences in light scattering detected.

This work has focused on whether differences in chromosomal structure and organization account, at least in part, for differential observations of the light scattering properties of tumor cells.

Through collaboration with another center member, Professor Vinayak Dravid, Dr. Backman was able to use sophisticated electron microscopy measurements of the surface roughness of cancer and normal cells to show that his results were not due to changes in the surface properties of tumor cells.

Instead, the changes in light scattering were emanating from components within the cell, very possibly the nucleus (where chromatin resides).

When the Backman group treated cells with a drug known to alter the compactness of chromatin they found not only a significantly increased read-out of genetic information in the chromosomes but also a noticeable change in light scattering properties.

These results provide the first physical evidence supporting the theory that changes in chromatin organization occur at a very early stage of cancer development.

We sketch out two recent attempts to move beyond the current approaches using both physics-informed and biology-informed perspectives. We hope that these two most recent (unpublished) projects will help us get a basic understanding of what cancer is and how it proceeds.

An Evolutionary Game In Space Between Cancer and Its Surroundings: How Does the Game Determine the Ensuing Resistance to Therapy?

Cancer is a complex condition with strong ecological components that consist of cancer cells as well as stromal cells, the cells making up the connective tissue surrounding a tumor.

Multiple myeloma (MM), a cancer of plasma cells that sequester in the bone marrow, is often fatal because emergence of chemotherapy-resistant MM cells is usually inevitable. Bone marrow stromal cells (ST) are primarily made up of fibroblasts, which are precursor cells that eventually develop into connective tissue. These cells play an important role in MM progression and chemotherapy response.

We use a microfluidic device, or "ecology," that mimics the bone marrow environment under chemotherapy on a very small scale in order to visualize how multiple myeloma and stromal cells interact and how resistance to chemotherapy can develop.

Components of the microfluidic ecology include extracellular matrix (connective tissue) and stromal cells, varying levels of chemotherapeutic drug, and weakly connected microhabitats.

By studying these conditions, we can propose models of how resistance emerges in the context of interaction between these factors within a given space – a concept called *spatial evolutionary game theory.*

Using this theory, we can take into account (i) fitness (i.e., cells' ability to survive) as a relationship between the different cell types within a population and the amounts of drug they are exposed to and (ii) migration of cells within the space as both random and survival-seeking movement.

Fig. 1A presents the experimental dynamics between MM and stromal cells in time and space in a complex microecology under chemotherapeutic "stress": the situation transforms from a competitive "prisoner's dilemma" (a game theory concept in which two given parties do not necessarily cooperate with each other) into a coordination game where the stromal cells help the cancer cells. This enables multiple myeloma and stromal cells to coexist within the drug gradient.

Fig. 1B shows that evolutionary game theory applied to two interacting cell populations can predict future densities of the two cell populations (e.g., bone marrow stromal and cancer cells) based on their fitness as a consequence of population make-up. This opens the possible clinical use of such analysis for predicting and controlling cancer progression.

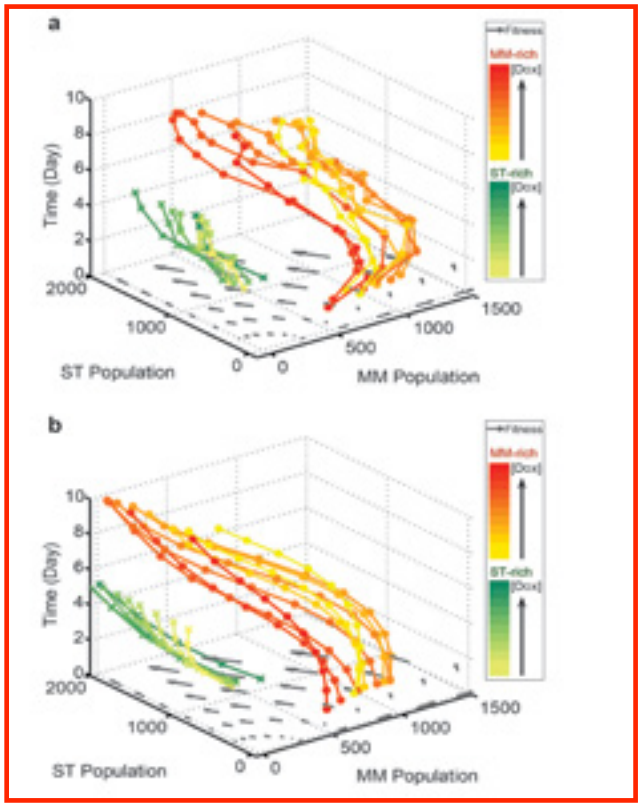


Figure 1: Populations of regions with different doxorubicin (chemotherapy) concentrations in a gradient. (a} Phase portrait of experimental data. (b} Phase portrait of game theory model

The Patterns of Mutations and Non-Mutations: Finding Haystacks as Well as Needles

In cancer, mutations in the genome do not show a random pattern. Using our microfluidic ecologies, we have evolved MM cells to achieve resistance 16 times greater than their original resistance to the chemotherapeutic drug doxorubicin within 2 weeks.

We have determined the DNA sequences of the resulting resistant MM cells and have found that although resistance emerged within only 2 weeks, the pattern of mutations clearly breaks up into regions of "hot spots" where the mutation rates are high and "cold spots" where the mutation rates are low.

It is, of course, easier to pinpoint where mutations occur (the needles in the haystack) and what role they may play in cancer progression, but much more difficult to look at a large region with low mutational density (the haystacks) and try to guess why that region does not necessarily show high mutation rates. However, the emergence of "cold spots'' implies that these regions contain critical genes that, if mutated, could seriously impact the fitness of the cell.

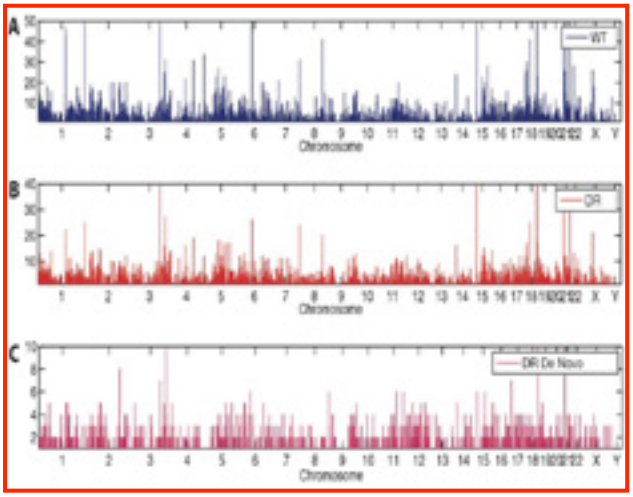


Figure 1. Distribution of mutations across whole genome. (a) Input MM cancer cell (WT), all mutations. (b) Resistant MM cells after 14 days within the gradient chip, all mutations. (c) De novo mutations from the resistant cells.

One approach to examining the cold spots is to annotate the highly expressed genes that are never mutated but are expressed at significantly different levels in evolved vs. wild-type (WT) (i.e., starting) cell populations. These are presumably important genes that cannot be mutated.

When we compared the expression levels of certain genes between evolved and WT cells, we found 417 genes that are expressed differently but never mutate in either WT cells or evolved cells grown on the chip. In other words, these genes are the mutational cold spots. Among them, 257 genes are up regulated or down-regulated more than 8-fold in evolved vs. WT. Interestingly, these non-mutated yet differentially expressed genes are mostly related to the most fundamental biological processes for cell survival: cell metabolism, synthesis of biological compounds, protein breakdown, and transport of molecules.

We also observe that non-mutated and up-regulated genes include heat shock proteins, which are important proteins that guide the proper formation of essential regulators of cell growth and enhance cell survival by maintaining damaged proteins in normal conformation.

Most fascinating in the non-mutated genes are genes that we consider to be "super-protected" since they are never mutated in either evolved or wild type cells: MAGEA6 and MAGEA8.

The MAGEA families play an important role in embryonic development as well as cellular differentiation in cancer cells and tumor growth, implying that ancient cellular pathways are protected in cancer.

The results shown here span ideas not only from the engineers, oncologists, biochemists, and genomic analysts of the Princeton PS-OC, but also from: Moffit PS-OC (Ariosto Silva and Robert A. Gatenby), ASU PS-OC (Paul Davies) and The Computer Science Department at Princeton (David Blei).

Using Physical and Mathematical Sciences to Understand the Fluid Phase (i.e., the Blood Circulation Phase) of Solid Tumors, Known as Carcinoma

Specifically, we are interested in the relationships between the primary tumor, the circulating tumor cells (CTCs) that escape the primary tumor, and the secondary tumors that develop when CTCs seed at distant sites – a phenomenon known as metastasis. This understanding will enable the use of blood samples from patients to predict how they might respond to treatment.

Prescribing the right treatment at the right time to the right patient can prolong a high quality of life, reduce side effects, and reduce hospital time.

Multiple characteristics of single rare cells from the solid and the fluid phases of carcinomas can be evaluated using one technique that identifies rare cells at the single-cell level. These rare cells can be analyzed directly from a patient sample that contains roughly 30 million cells. Multiple types of carcinomas are being studied with our rare cell high-content analysis (HCA) platform, including lung, prostate, pancreatic, liver, ovarian, breast and colon cancer. All research is conducted using patient blood samples, bone marrow and tissue.

Mathematical models of tumor cell spread from primary to metastatic sites can predict the different paths, which tumor cells could possibly metastasize within the body. Predictions based on these models have the potential to modify the current guidelines of cancer management.

Despite advancements in medical knowledge, we have failed to quantify our understanding of patterns of metastatic tumor spread to distant sites that could predict how CTCs move through the circulatory and lymphatic systems of cancer patients.

The Scripps PS-OC has developed mathematical models to understand metastatic cancer progression for many of the major solid tumor types, including primary lung, breast, and colorectal cancers.

These mathematical models categorize each metastatic site as either a `spreader' site (has a higher chance of spreading CTCs throughout the body) or a `sponge' site (has a higher chance of attracting CTCs from within the body). This allows us to group subpopulations of patients according to their combination of primary tumor plus first metastatic site.

While metastases to the adrenal gland are common in lung cancer, there is no clear consensus as to whether they occur through the lymphatic system or through the blood. Although the theory that metastasis to the adrenal gland occurs via the lymphatic system is not novel, this finding has yet to be commonly utilized in clinical practice.

Our mathematical models of primary lung cancer (Newton et al., 2013) reveal that in primary lung cancer, metastasis to the adrenal gland occurs as a very early event.

Furthermore, the adrenal gland also serves as a spreader site. By integrating this finding with existing literature, we can identify a subgroup of patients who might indeed benefit from a primary intervention, i.e., aggressive treatment of their adrenal metastases with surgery or radiation *(Bazhenova et al., 2014).*

CTC aggregates, also known as circulating tumor microemboli (CTM), can be identified and analyzed using a fluid biopsy. Combined with clinical data, it can provide a way to diagnose lung cancer.

We have routinely detected and characterized CTM in blood samples from lung cancer patients using the high-definition (HD) CTM assay, one of the assays in our rare cell HCA platform.

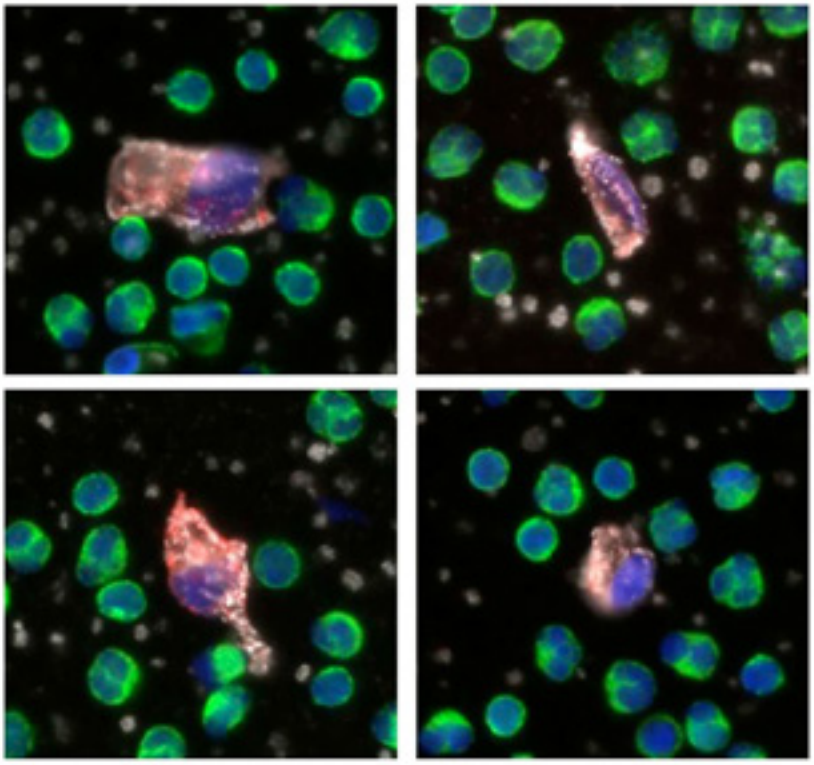
The CTM are commonly found in a wide range of sizes, some containing hundreds of cells.

We performed a prospective clinical study of patients with undiagnosed lung nodules that were undergoing diagnostic PET scans, an imaging test that uses a radioactive tracer to look for disease in the body (Nair et al., 2013).

Data from the HD-CTM assay were combined with patient clinical information and analyzed to create a predictive model, called LungDx, for the diagnosis of lung cancer. This model will be available to the public as a web-based application where clinicians can enter patient characteristics, imaging and HD-CTM assay results in order to obtain a probability that a given lung nodule is malignant. *(Continued on next page)*

LungDx has demonstrated its initial prognostic potential in non-small cell lung cancer, a traditionally difficult disease to diagnose and monitor. CTM proved to be highly specific in detecting cancerous versus benign nodules *(Carlsson et al., In preparation 2014).*

Our rare cell high-content analysis platform successfully detects CTCs in the blood of cancer patients and allows for the detection of other cell types.



The HD-CEC assay can accurately detect circulating endothelial cells (stained in red, white, and blue) in MI patients among the surrounded white blood cells (blue and green).

The endothelium constitutes the inner cellular lining of the blood vessels and the lymphatic system. The cells that form the endothelium are called endothelial cells. Normal adults have a small number of circulating endothelial cells (CEC) in peripheral blood.

Elevated levels of CECs occur in response to various pathological conditions such as a myocardial infarction (MI), i.e., a heart attack. We developed a new fluid biopsy technique that could identify patients at high risk of MI by identifying CECs as markers in the bloodstream.

This simple and non-invasive test, named the HD-CEC assay, has the potential to be a robust diagnostic biomarker to determine which patients are at highest risk of a heart attack.

High-content analysis of single cells isolated from patient samples uses both biophysical and genomic approaches to study the evolution of cancer.

A key advantage of the HCA platform is the ability to isolate single and grouped cells for further analysis including single-cell genomics.

Our single cell genomic and copy number variation analysis protocol has demonstrated the emergence of different cancer cell populations over time in response to therapy in a prostate cancer patient (Dago et al., Submitted 2014).

A better understanding of how cell populations differ within tumors is necessary to make full use of new therapies that target cancer at the molecular level.

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Highlights: Chris Rycroft

Chris Rycroft

I am an applied mathematician and from 2010–2013, I was an instructor in the UC Berkeley Mathematics Department. My background is in computer simulation and designing numerical techniques to model physical and mechanical systems.

Prior to my appointment at Berkeley, I worked on the mechanical properties of granular media (such as sand or salt), as well as deformation and fracture in amorphous metallic alloys.

I became involved with the Stanford PS-OC in 2010. I attended the annual PS-OC meetings, and despite having little training in the biological sciences, I became very interested in the questions that the PS-OC was trying to address, particularly on understanding how tumor cells interact with each other and their local environment. I got to know a number of the experimental groups in the Stanford PS-OC, and I became involved in several studies where I could use computer simulation to model and interpret experiments.

In one project, I worked with Prof. Jan Liphardt's group to understand whether interactions between mammary acini (the ring-like structures made up of breast epithelial cells that form mammary glands) may play a role in breast cancer development. We studied a model system where many acini are placed on a collagen gel and prepared so that they begin to disorganize (i.e., take on more invasive qualities).

The experiments showed that single acini disorganize at a statistically much lower rate than when multiple acini are nearby, confirming that acinus–acinus interactions may indeed be important. Furthermore, when multiple acini are present, collagen lines of higher density form between them, as shown by the intense red bands in Figure (B). The presence of the lines is linked to the rapid disorganization, and severing the lines with a laser removes the effect.

When I first joined the project, the process of collagen line formation was not yet understood. I expected that it was purely mechanical, based on similar patterns I had seen in metallic alloys.

Using a numerical method that I had recently developed, we simulated the process and showed that the lines arose from the specific mechanical properties of the collagen gel (Figure C, D).

This clarified results from the initial experiments and we subsequently used the simulations to test a variety of hypotheses about the acinar interactions. The experiments were published in Proc. Natl. Acad. Sci. in 2013, and we aim to publish a companion simulation paper in 2014.

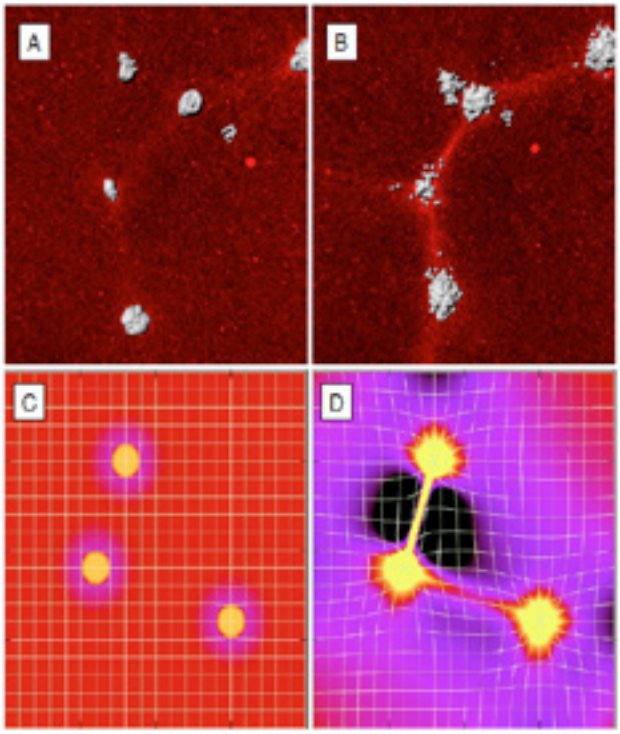


Figure caption: Experimental images of a model system to study mechanical interactions relevant to cancer development.
(A) Mammary acini (grey) are placed on a collagen gel and begin to disorganize. Fluorescent markers placed within the gel can be used to track its density.
(B) Bands of high-density collagen form between neighboring acini over time.
(C, D) Eulerian finite-difference simulations of the collagen gel motion, where colors indicate the relative collagen density (black: low, yellow: high). If the gel is modeled as a nonlinear elastic material, and the acini pull radially on the gel, high-density lines of collagen form between acini as a consequence of an asymmetry between the tensional and compressive response

Highlights: Matthew J. Paszek

Matthew J. Paszek

I was an active participant in the Stanford PS-OC from 2010 to 2013 as a post-doctoral fellow training with Valerie Weaver. Our work started with a computational model, which predicted that cell surface proteins, acting as physical entities, would influence how cell surface receptors organize themselves on the cell surface and send signals to the cell.

In a highly collaborative project that included clinicians, bioinformaticians, physicists, engineers, and cell biologists from within the Stanford network and across the globe, we tested the relevance of these predictions in cancer.

We discovered that aggressive cancers, including those presenting with circulating tumor cells (CTCs, i.e. cells that escape the primary tumor and travel through the bloodstream to distant sites), are associated with expression of unusually bulky cell surface glycoproteins, which are proteins with carbohydrate groups attached to them.

In essence, tumor cells are coated in a slimy layer, and our computational models predicted that this slime would be important in the functioning of tumor cells.

Consistent with model predictions, we found that these glycoproteins are organized in such a way that adhesion receptors called integrins are clustered into complexes, thereby triggering integrin signaling and consequently tumor cell growth and survival. This work has recently been accepted as a full report in the journal *Nature*.

During the project we developed new approaches for imaging and manipulating the cell surface at the microscopic level, built computational analysis tools for evaluating bulky glycoprotein signatures of cancer, conducted single molecule imaging experiments, and analyzed CTCs from the clinic.

Our work would not have been possible without the interdisciplinary framework provided by the PS-OC Program.

On a personal level, the unique PS-OC training environment facilitated my transition into my current independent faculty position in the School of Chemical and Biomolecular Engineering at Cornell University.

My lab continues to combine computational, super-resolution imaging, and cell biology approaches to unravel biophysical mechanisms of tumor progression.

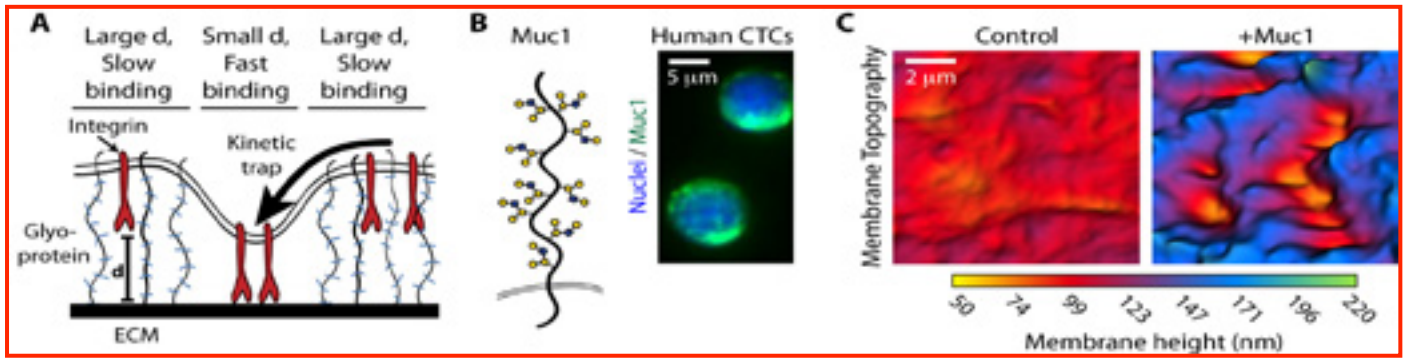


Figure: A) Computational models predict that bulky glycoproteins would facilitate integrin clustering by physically reshaping the dimensions of the cell-extracellular matrix (ECM) interface to establish a kinetic trap. B) We found that such bulky glycoproteins, like the mucin Muc1, were abundantly expressed on the surface of aggressive cancer cells, including CTCs. C) Expression of Muc1 altered the nanoscale topography of the cell-ECM interface, promoting integrin signaling and tumor cell growth and survival. (Paszek et al., Nature, accepted)

Marbles, Epigenetics and Drug Resistance

In the 1930s Conrad Waddington suggested that genetically identical cells could differentiate into a variety of cell types (e.g. neuronal, muscle, etc.) that form a living organism by navigating down different "landscapes."

For example, he pictured a cell as a marble moving down a mountain with many canyons. All of the cells at the top of the mountain are identical and stem-like (i.e., can differentiate into any cell type), but they become different depending on the canyon they follow (**Figure 1**).

Developmental biologists have extensively studied how stem cells differentiate into a living organism. Recently we have discovered that cancer cells also move along epigenetic landscapes. These landscapes are defined by special modifications of the DNA that do not change its sequence, but affect the way it is read. We have consequently been studying how these landscapes might impact the major challenge in cancer treatment – acquired drug resistance.

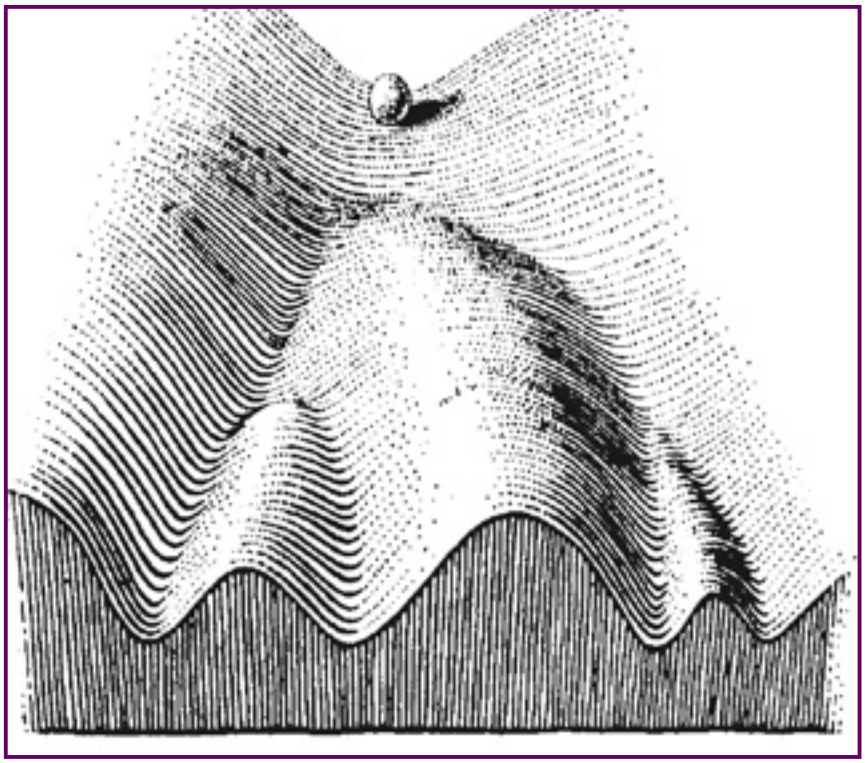
To examine this connection, we developed a series of drug-resistant lymphoma cells and then attempted to define the molecular changes responsible for that resistance.

We first looked at the genetic level and found there were no new relevant mutations (changes in DNA sequence) in the resistant cells. In contrast, the resistant cells displayed dramatic differences at the epigenetic level.

Using high throughput sequencing (a method to rapidly determine the sequences of large amount of DNA), we showed that the DNA methylation of the resistant cells was dramatically different than the original ones, suggesting that the cells had moved along the epigenetic landscape to become drug resistant.

Normal white blood cells undergo changes along epigenetic landscapes during their normal development: they start off as undifferentiated cells and eventually change into cells that can produce antibodies.

We hypothesize that the drug we were using caused cancer cells to move backwards – up the landscape – so that the resistant cells became more undifferentiated than the original sensitive cell.



The epigenetic landscape appears to be coupled to the degree to which cells are resistant to treatment; cells at the bottom of the canyon die rapidly when they are exposed to the drug, whereas cells at the top of the mountain are resistant.

We are currently testing these hypotheses using drugs that can specifically move cells up and down these landscapes in order to potentially make the resistant cells once again susceptible to treatment. In the clinic, these drugs may eventually be used to improve the effectiveness of chemotherapy and could ultimately reduce the chance of cancer recurrence.

Figure 1: Epigenetic landscape as imagined by Waddington in the 1930s

"Thoughts from an Oncologist"

by Mitchell Gross, MD, PhD

As a practicing oncologist, I am often frustrated by how helpless we are in the face of this terrible disease.

Despite technological innovations in high-content genetic analysis and an expanding list of molecularly targeted therapies, the clinical challenge presented by cancer patients remains daunting.

It is clear that new approaches are needed in order to adequately treat and care for our patients. Six years ago I became involved in the University of Southern California's nascent effort to link physical scientists and cancer biologists, hoping it might bring some new perspective and much-needed progress to the way we approach cancer.

I undertook the task of examining some of the basic 'tenets' of oncology that I had learned during my clinical training as part of the USC Physical Sciences-Oncology Center (USC PS-OC) effort.

For example, I was taught that chemotherapy is most effective when administered to the maximal tolerated dose over the shortest possible time.

I was amazed to learn that the theory behind this concept came from laboratory experiments conducted in the 1960s.

Despite countless laboratory and clinical studies conducted over the span of 50 years, very little progress had been made in refining our basic understanding of why chemotherapy works at all.

To that end, our team is committed to defining a "unified theory of chemotherapy;" a goal that is shared across many programs within the larger PS-OC Network.

In the ensuing years, I have gained confidence that such a goal is both attainable and will lead to major changes in the way cancer therapy is delivered and cancer is treated.

There is no question that the PS-OC Program has changed my perception of cancer. I now see it as the dynamic interplay between cell growth and division in response to environmental pressures brought on by the host.

I am inspired by our ongoing collaborations with physical scientists and the interest they display as they bring new insights and techniques to bear on the fundamental problems in oncology. I look forward to gaining a new understanding of cancer that will contribute to an improved ability to control – and even cure – more and more cancer patients in the years to come.

Mitchell Gross, MD, PhD
Associate Professor of Medicine
University of Southern California

TRAINING THE NEXT GENERATION: AMY BLATT

Tackling cancer is a long-term commitment, and training the next generation of researchers is a critical. This starts at the undergraduate level, by capturing the imagination of students and encouraging them to think creatively about a problem that has challenged scientists for decades.

When the Arizona State University PS-OC began 5 years ago, we set up an innovative undergraduate course for high achieving students from both physical sciences and life sciences backgrounds. The course runs annually in the Spring semester and students attend lectures by clinicians and top researchers, tour labs and undertake research-oriented projects.

Gratifyingly, many of the young people on the course have been inspired to pursue work experience and vacation internships in cancer research laboratories across the country.

One such star student is Amy Blatt. Amy was an Honors College sophomore in our class in 2012. When we introduced her to the PS-OC approach, she changed the focus of her part-time research and joined the lab of one of our investigators, Deirdre Meldrum, in ASU's state of the art Biodesign building.

Amy still works there part time during the semesters, helping to develop improved diagnostic tools for the detection of esophageal cancer. We have followed Amy's progress with pride. In the summer of 2012, before her junior year, she took part in a project at Massachusetts General Hospital, studying novel ways to image cancer cells, to test the efficacy of drugs that stop tumors forming blood vessels.

Amy's work resulted in a co-authored paper published in the Journal of Biomedical Optics in September 2013 and she was invited to present her work at a national conference.

The following summer, Amy returned to Boston, this time to join a National Cancer Institute funded initiative at the Broad Institute. There she worked on a theory project: constructing computational models to predict tumor weaknesses based on genomic sequencing. This forms part of a new generation of drug design directed to personalized treatments.

Amy is now about to graduate with a degree in biomedical engineering and armed with such impressive undergraduate research experience, she has been juggling offers for PhD programs, including a place at Cambridge University on one of the projects funded by Cancer Research UK.

"This was my first opportunity to learn about the complexities of cancer and interact with cancer researchers and clinicians from the Mayo Clinic. My positive experience from this class as well as an inherent interest to learn more about the disease led me to perform research in three different labs during my time as an undergraduate." – Amy Blatt, Undergraduate, Arizona State University

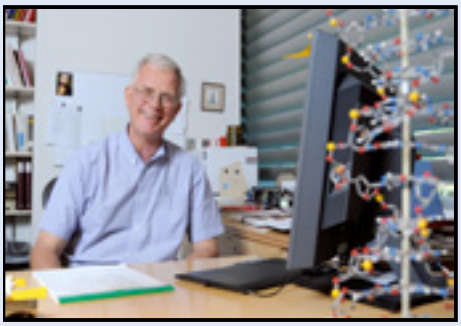
In four years of research at MIT, my colleagues and I have been trying to develop a finer understanding of cancer cell metabolism through the lens of cell mass accumulation. Participating in the PS-OC program has been one of the most beneficial parts of my scientific training, both for me as an individual and for our research. It provides a platform to interact with like-minded, interdisciplinary scientists whose fascination and input have helped inform and shape the trajectory of experiments, as well as bolstered my enthusiasm for exploring biology through physical science perspectives. Given the scarcity of conferences, programs, and funding which emphasize the interface of physics and biology research, the PS-OC Program represents a truly forward thinking environment.
Mark Stevens, Graduate Student, MIT PS-OC

The PS-OC Program encourages physicians and scientists to get out of their comfort zone. By hanging out with physicists, biomedical engineers, and mathematicians we have developed new ways of thinking about how to therapeutically target cancer cells. It has let us evolve to think about how cancer cells interact with their microenvironment. I am proud to call myself a cancer ecologist.
Kenneth J. Pienta, MD Investigator, Princeton PS-OC

Partnering with physicists, mathematicians and other members of the physical sciences community has been an inspiring opportunity. It has led me, as a physician and surgeon, to re-look at clinical problems in a new light. It is especially exciting when a mathematician with no medical background, for instance, is given a clinical problem and develops a model that not only makes complete sense in the context of patient care, but then actually pushes patient care in a new direction based upon that different perspective/understanding. My research project was initially inspired by work being conducted by the PS-OC and would not have come this far were it not for the support of the PS-OC.
Randolph Schaffer, MD, Scripps PS-OC, Surgeon, Scripps Clinic

The PSOC Program not only supported but also actively promoted questioning of fundamental assumptions that have been integral to cancer biology and therapy for decades. This resulted in a vigorous and much-needed critical re-examination of cancer as a complex dynamical system and exploration of the first principles and key data elements necessary to develop predictive models for optimizing treatment strategies.
Robert A. Gatenby, MD, Principal Investigator, Moffitt PS-OC

Joining the PS-OC Program is the most exciting adventure I have ever had. We feel lost, get hammered, and sort out the mess together. As physical scientists, we are naive but at the same time are not limited by textbooks, and appreciate having a chance to understand more about cancer using provocative approaches.
Amy Wu, Graduate Student, Princeton PS-OC



The PS-OC interaction has taught me that reductionism is not useful in cancer studies. One needs to understand the "social" behavior of cells in a tissue context to begging to understand cancer. Cell lines that grow in isolation are not useful models. On the other hand, I have also been surprised to learn of the degree of social behavior and specialization in simple bacterial colonies. Thus the "hydrogen atom" of cancer studies has to be a community of cells in an appropriate 3D context.
Stuart Lindsey, PhD, Department of Chemistry and Biochemistry, Arizona State University

I am a postdoctoral fellow at The Methodist Hospital Research Institute PS-OC where my research focuses on the use of mild hyperthermia to overcome mass transport tumor barriers and improve anti-tumor efficacy. The PS-OC Program has provided tremendous support through which I have acquired indispensable knowledge and tools that are important for cancer research. I have become an expert in live-animal imaging and currently lead the intravital imaging core, assisting researchers to develop therapeutic carriers most suitable for cancer treatment. The PS-OC Program has also connected me with valuable collaborators through the Young Investigators' meetings and travel awards, for which I am grateful.
Dickson Kirui, PhD, Postdoctoral Fellow, TMHRI PS-OC

On the Front Cover:

Figure 1: Bright field image of well-vascularized breast cancer tumor. Image by Dickson Kirui, PhD, TMHRI PS-OC.

Figure 2 : Intravital microscopy imaging of a mouse tomato red breast tumor with the vasculature visualized using FITC dextran. Image by Rita Serda, PhD and Enrica de Rosa, PhD, TMHRI PS-OC.

Figure 3: Intravital microscopy imaging of a mouse tomato red breast tumor with oregon green liposomes flowing in the blood vessels. Low molecular weight blue dextran has already leaked from the vessels. Image by Rita Serda, PhD and Kenji Yokoi, PhD, TMHRI PS-OC.

Figure 4: Breast cancer vessels labeled with green dextran. Image by Dickson Kirui, PhD, TMHRI PS-OC.

Perspectives *is produced by Teresa Schuessler, Mariam Eljanne, Katrina Theisz, Rachel Hoskins, Chevas Samuels and Pauline Davies.*

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